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Abstract: Background: Echocardiographic assessment of left ventricular (LV) size and function using area-based indices and volumetric estimates is not well established in horses. Objective: To report reference intervals and measurement variability for uni-, 2-, and 3-dimensional echocardiographic indices of LV size and systolic function in Warmblood horses and to provide proof of concept for allometric scaling of variables to body weight. Unidimensional indices were to be compared to area-based indices and LV volume estimates to establish their clinical use. Animals: Thirty healthy Warmblood horses and 70 Warmblood horses with a primary diagnosis of mitral regurgitation or aortic regurgitation. Methods: Echocardiographic indices of LV size and systolic function were measured using an existing echocardiography database. Weight-related variables were scaled to body weight (BWT). Reference intervals and measurement variability were calculated, the influence of valvular regurgitation on LV size and function was investigated and agreement between different variables for detection of reduced, normal, and increased LV size and systolic function was assessed. Results: Reference values for healthy Warmblood horses were reported. Measurement variability was sufficiently low for clinical use of all variables. Allometric scaling was effective to correct diastolic LV dimensions and cardiac output for differences in BWT. Various echocardiographic indices resulted in different conclusions regarding identification of LV enlargement and systolic dysfunction in healthy horses and horses with valvular regurgitation. Conclusions and clinical importance: Echocardiographic assessment of LV size and systolic function should include joint assessment of multiple uni- and multidimensional indices. Area-based or volumetric indices that reflect LV long-axis motion should be included.

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Echocardiographic assessment of left ventricular size and systolic function in Warmblood horses using linear measurements, area-based indices, and volume estimates: A retrospective database analysis

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[Correction added on 03 December 2020 after first online publication: The numeric data in the Tables have been corrected in this article.]

Abstract

Background: Echocardiographic assessment of left ventricular (LV) size and function using area-based indices and volumetric estimates is not well established in horses.

Objective: To report reference intervals and measurement variability for uni-, 2-, and 3-dimensional echocardiographic indices of LV size and systolic function in Warmblood horses and to provide proof of concept for allometric scaling of variables to body weight. Unidimensional indices were to be compared to area-based indices and LV volume estimates to establish their clinical use.

Animals: Thirty healthy Warmblood horses and 70 Warmblood horses with a primary diagnosis of mitral regurgitation or aortic regurgitation.

Methods: Echocardiographic indices of LV size and systolic function were measured using an existing echocardiography database. Weight-related variables were scaled to body weight (BWT). Reference intervals and measurement variability were calculated, the influence of valvular regurgitation on LV size and function was investigated and agreement between different variables for detection of reduced, normal, and increased LV size and systolic function was assessed.

Results: Reference values for healthy Warmblood horses were reported. Measurement variability was sufficiently low for clinical use of all variables. Allometric scaling was effective to correct diastolic LV dimensions and cardiac output for differences in BWT. Various echocardiographic indices resulted in different conclusions regarding identification of LV enlargement and systolic dysfunction in healthy horses and horses with valvular regurgitation.

Conclusions and Clinical Importance: Echocardiographic assessment of LV size and systolic function should include joint assessment of multiple uni- and

Abbreviations: 2D, 2-dimensional; AAD, aortic annular diameter; AMM, anatomical motion mode; ANOVA, analysis of variance; AR, aortic regurgitation; BWT, body weight; CI, confidence interval; CV, coefficient of variation; d, diastolic; FAC, fractional area change; FS, fractional shortening; HR, heart rate; IVS, interventricular septal thickness; LA, left atrium or left atrial; LAA, left atrial area; LAD, left atrial diameter; llx, left-parasternal long-axis view; LV, left ventricle or left ventricular; LVFW, left-ventricular free wall; LVID, left-ventricular internal diameter; lx, long axis; M-mode, motion mode; MR, mitral regurgitation; MWT, mean wall thickness; NSR, normal sinus rhythm; RC, repeatability coefficient; RR, R-R interval in the ECG; RWT, relative wall thickness; s, systolic; SD, standard deviation; sx, short axis; TR, tricuspid regurgitation; WB, Warmblood horse.

multidimensional indices. Area-based or volumetric indices that reflect LV long-axis motion should be included.

KEYWORDS
cardiology, equine, heart, quantification, ultrasound

1 | INTRODUCTION

Echocardiography is an indispensable diagnostic procedure in equine cardiology.[1,2] Two-dimensional (2D) gray-scale imaging and M-mode echocardiography provide the basis for noninvasive evaluation of cardiac structures, chamber dimensions, and chamber function. In particular, assessment of left-ventricular (LV) dimensions and function is a crucial part of every echocardiographic examination and provides important information on hemodynamics, LV remodeling, and severity of disease. Traditionally, LV wall thickness and LV internal diameter have been measured from a right-parasternal short-axis view at the chordal level using M-mode recordings. However, standardized placement of the M-mode cursor (ie, bisecting the LV at the chordal level, parallel to the mitral annulus) can be challenging and is difficult to verify in all 3 dimensions.[3-5] Such linear measurements of wall thickness may not be accurate estimates of LV mass, particularly if asymmetric thickening is present. Furthermore, these linear measurements of the LV minor dimension may not well describe true LV size, as asymmetric LV dilation and dimensional changes along the major axis of the ventricle are neglected.[4] Consequently, LV fractional shortening (FS), the most commonly used index of LV systolic function in horses calculated from the LV internal dimensions measured at end-diastole and peak-systole, only represents the relative shortening of the LV in 1 single dimension, disregarding the fact that the LV contracts in all 3 dimensions. It may lack accuracy when the LV does not contract synchronously or when the cursor line is not placed optimally. A previous study indicated that LV FS, as opposed to other echocardiographic indices, is not suitable to detect exercise-induced changes in LV function in horses.[6] Hence, reliance on this index as a single measurement of LV systolic function may be problematic.

Measures that consider the short-axis area and the length of the LV may be more accurate estimates of internal LV dimensions and LV mass. Area-based measurements are less sensitive to asynchronous wall motion and allow assessment of shortening in 2 dimensions. Volumetric indices of cardiac size and function, considering all 3 dimensions, can be calculated using a variety of geometrical models and are generally considered more accurate and least affected by altered chamber geometry,[4] but differences and limitations related to the geometrical model used need to be considered.[4,5,7] This group routinely uses area-based indices and volumetric estimates of LV size and function that have been reported in a variety of studies.[6,8-10] However, at most institutions, area- and volume-based measurements of LV systolic function are primarily used in research settings and they are not widely established for routine use in horses.

The goals of this study were to investigate the feasibility and measurement reliability of different echocardiographic modalities for assessment of LV size and mechanical function, to calculate reference intervals in a group of healthy horses, to provide "proof of concept" for using volumetric estimates of LV size and function by comparing dimensional measurements and functional indices between healthy horses and horses with heart disease, and to compare the conventional unidimensional indices of LV size and function to area-based indices and to LV volume estimates that are based on a combination of linear and area measurements of the LV. We hypothesized that LV areas and volumetric estimates of LV size and function can be easily measured, are sufficiently reliable, allow detection of LV enlargement and dysfunction in horses with heart disease and provide complementary information to the conventional linear indices.

2 | MATERIALS AND METHODS

2.1 | Study sample

The study sample was chosen retrospectively from the digital echocardiography database (GE EchoPAC, GE Healthcare, Glattbrugg, Switzerland) of the Equine Hospital of the University of Zurich and included horses that underwent an echocardiographic examination at the hospital over a 4.5-year period. Enrollment criteria were: Warmblood breed; age >2 years; presence of normal sinus rhythm; absence of cardiovascular disease (healthy group) or presence of mitral or aortic regurgitation as a primary diagnosis (diseased group); and the availability of a complete, standardized echocardiogram of adequate quality, with an ECG recorded simultaneously and performed by a single operator (CCS) on a digital echocardiography system (GE EchoPAC, GE Healthcare, Glattbrugg, Switzerland). Horses that were sedated prior to or during the echocardiographic examination were excluded from the study.

One hundred Warmblood horses fulfilled the inclusion criteria. Thirty horses were considered healthy based on medical history, physical examination, electrocardiography and transthoracic echocardiography. They included 12 females and 18 males with a body weight of 450-680 (570 ± 53) kg and an age of 6-23 (12 ± 4) years [range (mean ± SD)]. The remaining 70 horses had a primary diagnosis of MR (n = 42; 16 females, 26 males; 430-710 (579 ± 65) kg; 4-28 (13 ± 5) years) or AR (n = 28; 9 females, 19 males; 495-720 (582 ± 54) kg; 5-25 (17 ± 5) years); none of the horses was in congestive heart failure.

All animals had been examined within routine hospital procedures and had received adequate human care according to the ethical standards of the university. This study being based on a retrospective data analysis, a specific animal use protocol approved by the governmental authorities was not required.

2.2 | Echocardiography

All horses had undergone a complete echocardiographic examination according to a standardized protocol.[1] During the examination, all horses had been standing in a quiet room and restrained by an experienced handler. Transthoracic 2D echocardiography (2DE) had been performed using a high-end digital echocardiograph (GE Vivid 7 Ultrasound system, GE Healthcare, Glattbrugg, Switzerland) with a phased array sector transducer (M4S phased array transducer, GE Healthcare, Glattbrugg, Switzerland) working at frequencies of 1.7/3.6 MHz (octave harmonics) and at frame rates between 46.8 and 77.9 frames/s (fps). An ECG had been recorded simultaneously for timing purposes.

All recordings had been obtained in right-parasternal imaging planes. The LV had been imaged in 2D mode in a short-axis view at the chordal level and in a long-axis (4-chamber) view. For the latter view, the probe had been directed slightly ventrally, so that the entire ventricle, including the mitral valve annulus and the ventricular apex, was visible in its entirety throughout the cardiac cycle.[1] The left atrium had been imaged in a separate long-axis (4-chamber) view directed dorsally to image the left atrium in its entirety throughout the cardiac cycle. The aortic valve and ascending aorta had been imaged in a long-axis view of the left ventricular outflow tract.

In each imaging plane, at least 3 representative, nonconsecutive cardiac cycles had been recorded. All images had been stored as 2D cine-loops in digital raw-data format (GE EchoPAC, GE Healthcare, Glattbrugg, Switzerland).

2.3 | Measurements

All measurements were performed offline by a single observer (DJB) using the digital raw-data image files (GE EchoPAC, GE Healthcare, Glattbrugg, Switzerland). Three nonconsecutive, randomly chosen cardiac cycles of adequate quality were measured for each imaging plane. Cycles immediately following an incident of 2nd degree atrio-ventricular block were excluded from analyses. In horses, in which the available recordings did not contain a sufficient number of complete cardiac cycles for all imaging planes, all available cycles were measured. On some recordings, insufficient image quality prevented unambiguous identification of anatomical landmarks for measurements on 3 nonconsecutive cycles; in those cases, only the cycles were measured in which the landmarks could be clearly identified. The HR of each measured cycle was calculated based on the RR interval preceding the analyzed cycle (HR = 60′000/RR).

The echocardiographic variables measured for the purpose of this study are listed in Appendix S1. In summary, the following measurements were performed[1,3,4,6,7,11-13]:

The peak-systolic aortic annular diameter (AAD) in a right-parasternal long-axis left ventricular outflow tract view, as an internal reference for body size; maximum left atrial (LA) dimensions in a right-parasternal long-axis 4 chamber view, optimized to image the LA, for calculation of LA-to-LV-dimensional ratios; linear measurements of LV size and function in 2DE-derived anatomic M-mode (AAM) recordings in a right-parasternal short-axis view at the chordal level; area measurements of LV size and function in a right-parasternal long-axis 4-chamber view optimized to image the LV and in a right-parasternal short-axis view at the chordal level, respectively; and volumetric estimates of LV size and function in a right-parasternal long-axis 4 chamber view optimized to image the LV, calculated using the modified (single plane) Simpson's model of discs, the area-length model, and the bullet model, respectively.

The LA dimensions, the LV internal chamber dimensions at end-diastole, and cardiac output were corrected for differences in body weight using the principles of allometric scaling.[11,14-16] Specifically, the measurements were normalized to a body weight (BWT) of 500 kg using the following equations: diameter (500) = measured diameter/$BWT^{1/3} \times 500^{1/3}$; area (500) = measured area/$BWT^{2/3} \times 500^{2/3}$; volume (500) = calculated volume/BWT × 500. In addition, linear indices were indexed to AAD, area measurements were indexed to AAD^2, and volumetric estimates were indexed to AAD^3.

Grading of severity of valvular regurgitation (trace, trivial, mild, moderate, severe) was achieved using a scoring system based on the number of imaging planes in which the high-velocity jet could be observed, the duration of the regurgitant signal, and the high-velocity jet area relative to the receiving chamber.[17] Thereby, regurgitation was classified trivial when the regurgitant signal was visible in 1 or more imaging planes, but was not present throughout systole and diastole and, at its largest, occupied >1/8 to 1/4 of the 2D area of the LA or LVOT, respectively, when assessed subjectively. Regurgitation was classified more than trivial (ie, mild, moderate, or severe) when it was easily located from multiple imaging planes and was present throughout systole or diastole. A regurgitant signal was classified mild when it occupied from 1/4 to 1/2 of the LA or LVOT; moderate when it occupied >1/2 to 3/4 of the LA or LVOT; and severe when it occupied >3/4 of the LA or LVOT, respectively. The horses were grouped according to the primarily affected valve, as judged by the clinician performing the echocardiogram (CCS).

2.4 | Data analysis and statistics

Data collection, graphical presentation, data analysis, and statistics were performed using commercially available computer software.

To establish reference intervals for the measured and calculated variables, we included the data of the 30 healthy horses. A dedicated software package was used (Microsoft Excel, Microsoft Corporation, Santa Rosa, CA; Reference Value Advisor v2.1, National Veterinary

School, Toulouse, France). Distribution of the data was checked using raw data box-and-whisker plots, histograms, and normal probability plots. The lower and upper limits of the reference intervals were calculated as mean ± $t_{(n-1)}$ × SD, with $t_{(n-1)}$ being the critical value of a *t*-distribution with n-1 degrees of freedom and for a 95% prediction interval and SD being the standard deviation. For normally distributed variables, untransformed data were used. If normal distribution could not be assumed, the reference interval was calculated based on Box-Cox transformed data. If individual outliers were identified by the software, they were generally retained in the analyses unless they were clearly identified as aberrant observations (ie, single, clearly isolated outliers originating from echocardiographic recordings of borderline quality), in which case they were excluded from calculations. The 90% confidence intervals (CI) of the limits of the reference intervals were determined using a bootstrap method.

The relationship of echocardiographic variables obtained in healthy Warmblood horses to BWT was assessed using linear regression analyses (GraphPad Prism v5.02 for Windows, GraphPad Software, La Jolla, CA). Where available, both raw data and weight-corrected data were included in linear regression analyses in order to assess the effect of weight correction.

The echocardiographic data of the healthy horses were compared to the data obtained on horses with a primary diagnosis of trivial-to-mild MR and moderate-to-severe MR, and on horses with a primary diagnosis of trivial-to-mild AR, moderate AR, and severe AR, respectively, using 1-way analysis of variance (ANOVA) with Dunnett's post hoc test (GraphPad Prism v5.02 for Windows, GraphPad Software, La Jolla, CA). The groups of moderate MR and severe MR were pooled, because the database contained only 1 horse with severe MR that fulfilled all inclusion criteria. Homogeneity of variances was assessed by graphical display of the data and validity of the normality assumption was confirmed by assessment of normal probability plots of the residuals. For each group, summary statistics (mean ± SD) were calculated.

To determine the intraobserver and interobserver measurement variability, a subgroup of 10 randomly selected horses (5 healthy horses and 5 horses with cardiac disease) was remeasured by the same observer (DJB) and by a second observer (CCS) on different days. Both observers were blinded to signalment, diagnosis, previously measured cycles, and previous measurements. Measurement variability was quantified using the within-subject variance for repeated measurements (residual mean square) determined by 1-way ANOVA with horses as the groups.[18] The within-subject SD (s_w) was calculated as the square root of the residual mean square (SigmaPlot v12.5, Systat Software, Inc., San Jose, CA). Variability was reported in 2 ways: The within-subject coefficient of variation (CV) was calculated as CV = s_w/mean × 100 and expressed as a percent value. In addition to the CV, the repeatability coefficient (RC, ie, the absolute value below which the difference between 2 measurements will lie with 95% probability) was estimated following the British Standards Institution recommendations as follows: RC = 1.96 × √2 × s_w = 2.77 × s_w.[18] The RC was reported to provide a clinically applicable measure of variability, hence an absolute value that allows comparison with measured changes in echocardiographic variables on a case-by-case basis. When applied clinically, the magnitude of change observed in a variable that is measured on 2 different occasions can be put in relation to the RC, to judge whether the change might be simply due to measurement error (observed change ≤ RC) or whether the change might represent a true change (observed change > RC). Summary statistics (mean ± SD) of all variables were calculated based on the first measurements of each horse (n = 10) and were reported for reference.

For comparison of different methods of measurement, a subset of core measurements (ie, indices of LV size at end-diastole, relative wall thickness, mean wall thickness, and fractional ejection phase indices) was chosen and analyzed. The relationship between different indices of LV dimensions and systolic function was assessed using linear regression analyses (GraphPad Prism v5.02 for Windows, GraphPad Software, La Jolla, CA). Bland-Altman analyses were performed to calculate mean bias and 95% limits of agreement for variables representing the same LV dimensional or functional index but being calculated using different methods (GraphPad Prism v5.02 for Windows, GraphPad Software, La Jolla, CA).[19,20]

The number of horses in which different methods of measurement obtained during a single examination revealed discordant results concerning LV dimensions and systolic function (ie, 1 variable indicated normal LV size and another variable indicated LV enlargement) was expressed as proportion and percentage for a variety of combinations. Agreement of different indices for detection of reduced, normal, and increased LV size (ie, classification agreement as judged based on the calculated reference intervals) in all horses and in horses with valvular regurgitation, respectively, was quantified using weighted kappa (κ_w) statistics (GraphPad, QuickCalcs, Online Calculator, www.graphpad.com/quickcalcs, GraphPad Software, La Jolla, CA). Thereby, κ_w > 0.75 indicated excellent agreement, κ_w ranging from 0.40 to 0.75 indicated fair to good agreement, κ_w ranging from 0 to 0.39 indicated poor agreement and κ_w < 0 indicated worse agreement.[21]

The level of significance for all statistical analyses was $P \leq .05$.

3 | RESULTS

The summary statistics, reference intervals and relationship to BWT of echocardiographic variables of LV size and function are shown in Table 1. Linear regression analyses showed that HR, AAD, LAD_{max}, LAA_{max}, $LVFW_s$, $LVIA_d$, $LVIV_d$ S, CO S, CO AL, and CO B were significantly positively related to BWT. After allometric scaling to a standard BWT of 500 kg and to AAD, respectively, LAD_{max}, LAA_{max}, $LVIA_d$, $LVIV_d$ S, CO S, CO AL, and CO B were not significantly related to BWT anymore.

Table 2 shows the comparison of echocardiographic variables of aortic dimension, LA size, and LV size and function in healthy horses and horses with various degrees of MR and AR, respectively. The AAD was not affected by MR but was significantly enlarged with severe AR. Left atrial dimensions were significantly affected by moderate-severe MR but not by AR. Most linear, area-based and volumetric indices of LV internal dimensions were increased with moderate-severe MR and with moderate and severe AR, respectively.

Both M-mode-based and 2D area-based RWT measurements were decreased with moderate-severe MR and with severe AR. M-mode-based MWT was unaffected by valvular regurgitation but 2D area-based MWT was decreased with moderate-severe MR. M-mode-based LV FS and 2D short-axis area-based LV FAC and LV EF B were not significantly affected by valvular regurgitation, but 2D long-axis

TABLE 1 Summary statistics, reference intervals, and linear regression analysis of variables used for measurement of LV size and systolic function in healthy Warmblood horses

	Summary statistics					Reference intervals		Linear regression (body weight)	
Variable	Unit	n	Mean	Median	SD	Lower limit of reference interval (90% CI)	Upper limit of reference interval (90% CI)	R^2	*P* value
Heart rate									
HR	bpm	28	40	39	6.2	27 (23.8-30.9)	53 (49.4-55.9)	0.34	.001[a]
Aortic dimensions									
AAD	cm	29	6.9	6.9	0.37	6.1 (5.96-6.33)	7.7 (7.47-7.87)	0.369	<.001[a]
Measurements of LA size									
LAD_{max}	cm	29	12.7	12.6	0.66	11.4 (11.03-11.8)	14.1 (13.7-14.5)	0.224	.008[a]
LAD_{max}/AAD	–	29	1.814	1.816	0.117	1.571 (1.506-1.637)	2.057 (1.99-2.121)	0.03	.36
LAD_{max} (500)	cm	28	12.2	12.2	0.49	11.1 (10.84-11.41)	13.2 (12.92-13.45)	0.005	.73
LAA_{max}	cm^2	28	109.7	110.4	7.14	94.7 (91.01-99.15)	124.6 (120.65-128.54)	0.615	<.001[a]
LAA_{max}/AAD^2	–	29	2.299	2.299	0.221	1.839 (1.720-1.951)	2.76 (2.647-2.897)	0.009	.61
LAA_{max} (500)	cm^2	30	100.5	100.8	5.52	89.0 (86.44-92.12)	112.0 (109.34-114.64)	0.017	.49
Linear measurements of LV size and function (M-mode, short axis at chordal level)									
IVS_d	cm	30	3.0	2.9	0.26	2.4 (2.29-2.56)	3.5 (3.35-3.64)	0.005	.72
$LVID_d$	cm	30	12.0	11.6	0.89	9.7 (9.26-10.2)	13.4 (12.87-13.88)	0.005	.7
$LVID_d$/AAD	–	30	1.667	1.655	0.164	1.327 (1.241-1.412)	2.007 (1.914-2.103)	0.129	.05
$LVID_d$ (500)	cm	30	11.1	11	0.89	9.2 (8.78-9.7)	12.9 (12.46-13.45)	0.1	.09
$LVFW_d$	cm	30	2.6	2.5	0.39	1.8 (1.59-2.01)	3.4 (3.22-3.65)	0.06	.19
IVS_s	cm	30	4.5	4.5	0.42	3.6 (3.43-3.89)	5.4 (5.18-5.65)	0.006	.68
$LVID_s$	cm	30	6.9	6.9	1.05	4.7 (4.16-5.34)	9.1 (8.58-9.63)	0.008	0.64
$LVFW_s$	cm	30	4.7	4.6	0.53	3.6 (3.27-3.89)	5.8 (5.48-6.03)	0.151	.03[a]
LV FS	%	30	40	39	6.4	27 (23.0-30.4)	54 (50.4-56.7)	0.035	.32
RWT_d	–	29	0.487	0.479	0.063	0.357 (0.322-0.389)	0.617 (0.582-0.651)	0.005	.72
MWT_d	cm	30	2.8	2.8	0.24	2.3 (2.16-2.42)	3.3 (3.15-3.43)	0.027	.38
$LAD_{max}/LVID_d$	–	30	1.102	1.1	0.088	0.919 (0.869-0.967)	1.284 (1.237-1.335)	0.081	.13
Area measurements of LV size and function (2D echocardiography, long axis)									
$LVIA_d$	cm^2	29	183.7	180.6	15.33	151.8 (143.12-160.65)	215.7 (206.87-223.82)	0.141	.04[a]
$LVIA_d/AAD^2$	–	30	3.861	3.822	0.487	2.848 (2.604-3.114)	4.873 (4.596-5.127)	0.106	.08
$LVIA_d$ (500)	cm^2	29	168.8	168.5	12.99	141.7 (135.03-149.29)	195.8 (188.61-203.34)	0.068	.17
$LVIA_s$	cm^2	29	81.4	80.1	11.60	57.2 (51.17-63.84)	105.6 (99.71-112.47)	0.064	.18
LV FAC	%	30	55	55	5.0	45 (42.8-47.9)	66 (63.2-68.0)	0	.99
$LAA_{max}/LVIA_d$	–	30	0.594	0.599	0.060	0.468 (0.436-0.501)	0.719 (0.685-0.753)	0.106	.08
Area measurements of LV size and function (2D echocardiography, short axis)									
$LVI_{sx}A_d$	cm^2	28	94.6	97.1	11.83	69.9 (119.30-76.79)	119.3 (112.05-126.08)	0.041	.28
$LVI_{sx}A_d/AAD^2$	–	29	1.995	1.949	0.35	1.266 (1.068-1.462)	2.725 (2.513-2.917)	0.077	.14
$LVI_{sx}A_d$ (500)	cm^2	30	89.4	89.9	13.89	60.5 (53.16-67.98)	118.3 (110.24-125.91)	0.035	.32
$LVI_{sx}A_s$	cm^2	29	32	31.2	8.38	14.5 (10.36-19.38)	49.4 (45.04-53.92)	0.005	.73
LV_{sx} FAC	%	30	67	66	5.9	54 (51.4-57.7)	79 (76.1-82.1)	0.005	.7
RWT_d A_{sx}	–	30	0.495	0.486	0.063	0.365 (0.332-0.400)	0.625 (0.588-0.660)	0.001	.88
MWT_d A_{sx}	cm	30	2.7	2.7	0.21	2.3 (2.15-2.41)	3.2 (3.05-3.26)	0.058	.2

(Continues)

TABLE 1 (Continued)

Variable	Unit	Summary statistics n	Mean	Median	SD	Reference intervals Lower limit of reference interval (90% CI)	Upper limit of reference interval (90% CI)	Linear regression (body weight) R^2	*P* value
Volumetric estimates of LV size and function (2D echocardiography)									
Modified (single plane) Simpson's (S) model of discs									
$LVIV_d$ S	mL	29	1475	1438	200.7	1057 (939.4-1172.9)	1893 (1795.7-2009.3)	0.13	.05[a]
$LVIV_d$ S/AAD^3	–	30	4.504	4.394	0.891	2.651 (2.177-3.160)	6.357 (5.870-6.848)	0.091	.1
$LVIV_d$ S (500)	mL	29	1296	1290	164.1	954 (862.9-1045.7)	1638 (1537.4-1710.5)	0.055	.21
$LVIV_s$ S	mL	28	412	415	81.6	241 (200.5-291.4)	582 (540.4-624.9)	0.068	.17
LV EF S	%	30	71	70	4.9	61 (59.1-63.6)	81 (79.1-82.8)	0	.96
SV S	mL	28	1065	1042	139.2	774 (702.1-854.8)	1356 (1277.7-1434.1)	0.112	.07
CO S	L/min	25	41.8	43.3	7.91	25.1 (19.83-29.82)	58.4 (53.39-63.02)	0.313	.002[a]
CO S (500)	L/min	25	37.1	37.0	6.02	24.4 (20.93-28.35)	49.7 (46.13-53.06)	0.003	.81
Area length (AL) model									
$LVIV_d$ AL	mL	29	1542	1504	216.2	1091 (967.7-1211.1)	1992 (1871.0-2099.6)	0.116	.07
$LVIV_d$ AL/AAD^3	–	29	4.625	4.518	0.843	2.869 (2.428-3.350)	6.381 (5.901-6.821)	0.096	.1
$LVIV_d$ AL (500)	mL	29	1356	1365	177.3	987 (891.3-1080.5)	1726 (1620.6-1827.4)	0.058	.2
$LVIV_s$ AL	mL	29	424	423	102.5	210 (152.5-264.2)	637 (583.4-692.0)	0.049	.24
LV EF AL	%	30	72	72	5	62 (59.2-64.0)	83 (79.9-85.4)	0	.99
SV AL	mL	28	1132	1108	154.6	809 (722.1-900.9)	1454 (1361.7-1533.2)	0.109	.08
CO AL	L/min	25	44.6	45.5	8.03	27.6 (22.84-33.03)	61.5 (56.37-65.86)	0.308	.002[a]
CO AL (500)	L/min	25	39.5	39.2	6.05	26.8 (23.31-30.76)	52.3 (48.64-55.60)	0.001	.91
Bullet (B) model									
$LVIV_d$ B	mL	28	1466	1492	189.9	1069 (959.4-1175.9)	1862 (1756.8-1975.4)	0.073	.19
$LVIV_d$ B/AAD^3	–	30	4.600	4.479	1.091	2.794 (2.500-3.155)	7.274 (6.386-8.272)	0.094	.1
$LVIV_d$ B (500)	mL	29	1316	1326	203.0	893 (779.6-1002.6)	1739 (1620.4-1847.6)	0.043	.3
$LVIV_s$ B	mL	28	349	346	89.0	163 (112.1-213.5)	534 (481.8-583.0)	0.019	.47
LV EF B	%	30	76	76	4.8	66 (63.1-68.4)	86 (83.3-88.1)	0.002	.8
SV B	mL	28	1117	1119	147.8	808 (726.9-898.9)	1426 (1339.7-1506.5)	0.099	.09
CO B	L/min	27	44.8	43.6	8.42	27.1 (22.21-32.13)	62.4 (57.36-67.03)	0.446	<.001[a]
CO B (500)	L/min	27	40.0	39.6	6.80	25.8 (22.05-30.06)	54.3 (50.36-57.85)	<0.001	.96

[a]Significant positive relationship to BWT. For detailed explanation of echocardiographic indices see Appendix S1.
Abbreviations: CI, confidence interval; n, number of horses; SD, standard deviation.

area-based LV FAC, LV EF S and LV EF AL were significantly decreased with trivial-mild and moderate-severe MR and with trivial-mild AR. All stroke volume indices were significantly increased with moderate and severe AR, whereas cardiac output indices were only increased with severe AR.

The intraobserver and interobserver measurement variability of all variables is summarized in Table 3. The coefficient of variation was <15% for all echocardiographic indices, except for the intraobserver variability of $LVIV_d$ B/AAD^3 (CV = 16.2%). Fifty-four of 59 indices had an intraobserver CV < 10%; all 5 indices with a CV ≥10% (range, 10.3%-16.2%) were indices that were allometrically scaled to AAD. Forty-six of 59 indices had an interobserver CV < 10%; of the 13 indices with a CV ≥10%, 7 were indices calculated using the modified Simpson's model of discs (range, 10.0%-12.9%) and 3 were indices calculated using the area-length model (range, 10.0%-13.5%). The average of the intraobserver and interobserver variability was lower for linear variables (2.5% and 3.0%) compared to area-based variables (4.7%, 5.8% and 5.7%, 4.6%) and volumetric variables (5.3%-8.2% and 6.4%-9.3%) (Supporting Information Table S1).

Supporting Information Figures S1-S5 summarize the agreement of different echocardiographic variables used for assessment of LV size, LV wall thickness, and LV systolic function. Table 4 shows the proportions and percentages of horses in which different methods of measurement obtained during a single echocardiographic examination revealed discordant results concerning LV size, LV wall thickness, and LV systolic function. Classification agreement, quantified by weighted

Kappa, was largely independent of the study sample under investigation (ie, all horses vs. horses with valvular regurgitation only). Agreement was fair to excellent for all indices of LV internal dimensions at end-diastole and for MWT but was poor for RWT. Agreement of area- and volume-based ejection phase indices of LV systolic function (ie, FAC, EF) with LV FS was poor or worse and with LV_{sx} FAC it was mostly poor. Agreement of EF indices with LV FAC was fair to excellent.

4 | DISCUSSION

This study defines reference intervals and describes measurement variability for a multitude of uni-, 2-, and 3-dimensional echocardiographic indices of LV size and function in Warmblood horses. It further provides proof of concept for the use of area-based variables and 2DE-based volumetric estimates of LV size and function in horses.

Table 1 summarizes reference intervals for a comprehensive set of variables of LV size and function. Other studies in horses demonstrated that LA and LV dimensions are significantly related to BWT,[14-16,22-26] but detailed comparisons of these studies are difficult because of differences in study samples in regard to breed, age range, and range of body weight. In one study, aortic root diameter, LVIDd, LVIDs, and LVFWs were significantly related to BWT in adult Standardbreds,[23] whereas in another study little or no linear correlation was found between any of the linear LV dimensions and BWT within a group of large horses of different breeds.[26] To our knowledge, the relationship of LV areas and volumes to BWT, taking LV long-axis dimensions into consideration, has not been previously investigated in horses. In this study, a significant, weak to moderate influence of BWT was demonstrated for AAD and for maximum LA dimensions (which were reported for comparison). A significant but very weak effect of BWT was shown for diastolic LV dimensions that were derived from long-axis measurements (ie, $LVIA_d$, $LVIV_d$ S), but not for those that were derived from short-axis measurements (ie, $LVID_d$, $LVI_{sx}A_d$, $LVIV_d$ B). This suggests that body size influences cardiac long-axis dimensions to a larger extent than short-axis dimensions. A significant influence of BWT was not shown for peak-systolic LV dimensions, fractional changes of LV internal dimensions, and measurements of LV wall thickness (with the exception of $LVFW_s$). This can be explained by a lack of power to detect relatively small weight-related alterations within the study sample, but also suggests that weight-related differences may not be clinically relevant for these variables. Cardiac output was significantly but weakly influenced by BWT; as CO = SV × HR and since SV was not significantly related to BWT, HR appeared to be the major determinant of higher cardiac output with higher body weight. This was somewhat unexpected, since HR was previously shown to be inversely related to body mass in horses and ponies of different breeds, ranging from 46 to 1018 kg.[27] However, the present study sample only comprised Warmblood horses with a relatively narrow range of body weights (450-680 kg). Part of the differences in body weight might in fact have been related to differences in body condition as opposed to true differences in body size, which could explain the positive correlation of HR to body weight.[28,29] This is purely hypothetical however, because body condition was not assessed in this study sample.

Consequently to these findings, for further comparisons, all diastolic LV dimensions and CO were allometrically scaled to correct for differences in BWT, based on the assumption that cardiac volumes are linearly related to $BWT^{2/3}$ (proportional to body surface area), and linear dimensions are linearly related to $BWT^{1/3}$ (proportional to body length).[14,15,30,31] Similar to previous studies, a scaling approach was chosen that corrects echocardiographic variables to a standard body weight of 500 kg and allows intuitive interpretation of weight-corrected variables.[8,9,16] In addition, a second method was applied to correct for different body size by indexing LV dimensions to AAD, under the assumption that aortic annular dimensions are directly related to BWT (which was confirmed by the results of this study), are little affected by alterations in stroke volume and blood pressure, and can therefore serve as an internal reference for body size in lack of an accurate body weight.[11,14,16,30]

Overall, the results of this study showed that allometric scaling of echocardiographic measurements of diastolic LV dimensions and CO in Warmblood horses is effective and eliminates the significant influence of BWT on these variables (Table 2). Hence, normalization of measurements of diastolic LV size and CO to a BWT of 500 kg provides a clinically applicable and intuitive method for weight correction in Warmblood horses. As indicated by the data, indexing diastolic LV dimensions to AAD might not be as sensitive to detect LV enlargement as scaling to a standard BWT.

Noticeably, even the diastolic LV dimensions that were not related to BWT remained unaffected by BWT after allometric scaling. Therefore, for consistency, further comparisons were conducted using the native and the allometrically scaled data for all diastolic LV variables.

It is important to notice that in the absence of respective data, allometric scaling must not be applied to correct for differences in BWT across different equine breeds. Another limitation to consider is the potential impact of body condition on the BWT-based scaling approach. Theoretically, the use of the ideal body weight as opposed to the actual body weight might result in better correction for differences in BWT. However, the ideal body weight can only be estimated by approximation, which would be an additional source of error. Finally, indexing of echocardiographic variables to AAD might not be valid for horses with aortic valve disease, because dilatation of the aortic root is expected in horses with moderate to severe aortic regurgitation.[16,32]

Progressive mitral and aortic regurgitation are associated with LA and LV volume overload, with the degree of chamber enlargement depending on the severity of valvular regurgitation.[32-35] Therefore, in the absence of a gold standard for quantification of LV size and function, comparison of echocardiographic variables between healthy horses and horses with different severities of valvular regurgitation allows assessment of the variables' relative clinical value to detect disease-related alterations. The results of this study provide proof of

TABLE 2 Echocardiographic variables obtained in healthy Warmblood horses and in Warmblood horses with valvular regurgitation

			Mitral regurgitation (mean ± SD) (*P* value post hoc test)			Aortic regurgitation (mean ± SD) (*P* value post hoc test)			
Variable	Unit	Healthy (mean ± SD)	*P* value *F*-test (ANOVA)	Trivial-mild	Moderate-severe	*P* value *F*-test (ANOVA)	Trivial-mild	Moderate	Severe
n	–	30		22	20		8	9	11
Age	y	12 ± 4		13 ± 5	12 ± 6		14 ± 5	16 ± 6	20 ± 4
BWT	kg	570 ± 53		589 ± 74	569 ± 55		590 ± 40	584 ± 70	573 ± 20
HR	bpm	42 ± 8		43 ± 14	40 ± 7		40 ± 5	38 ± 6	41 ± 5
Aortic dimensions									
AAD	cm	7.0 ± 0.47	0.29	7.0 ± 0.47	7.0 ± 0.34	**.006**	6.9 ± 0.51 .92	7.5 ± 0.30 .06	7.5 ± 0.76 **.013**
Measurements of LA size									
LAD_{max}	cm	12.7 ± 0.74	**<.001**	12.8 ± 1.16 .95	13.9 ± 1.18 **<.001**	.065	13.0 ± 0.86	13.5 ± 0.58	13.3 ± 1.40
LAD_{max}/AAD	–	1.827 ± 0.135	**<.001**	1.792 ± 0.193 .69	2.007 ± 0.191 **.001**	.3	1.902 ± 0.092	1.804 ± 0.104	1.799 ± 0.118
LAD_{max} (500)	cm	12.2 ± 0.62	**<.001**	12.1 ± 1.00 .97	13.4 ± 1.10 **<.001**	.05	12.3 ± 0.73	12.8 ± 0.48	12.7 ± 1.14
LAA_{max}	cm^2	109.6 ± 9.60	**<.001**	111.9 ± 15.68 .77	128.4 ± 16.73 **<.001**	.23	107.8 ± 11.66	117.1 ± 11.41	116.2 ± 20.40
LAA_{max}/AAD^2	–	2.278 ± 0.247	**<.001**	2.232 ± 0.418 .87	2.685 ± 0.466 **<.001**	.14	2.303 ± 0.186	2.109 ± 0.283	2.119 ± 0.270
LAA_{max} (500)	cm^2	100.5 ± 5.52	**<.001**	100.7 ± 12.63 1	118.1 ± 14.86 **<.001**	.09	96.5 ± 8.58	105.8 ± 9.03	106.1 ± 17.28
Linear measurements of LV size and function (M-mode, short axis at chordal level)									
IVS_d	cm	3.0 ± 0.26	.26	2.9 ± 0.33	2.8 ± 0.42	.84	3.0 ± 0.29	3.1 ± 0.49	3.0 ± 0.45
$LVID_d$	cm	11.6 ± 0.89	**.002**	11.9 ± 1.27 .33	12.6 ± 0.80 **<.001**	**<.001**	11.9 ± 0.91 .8	13.4 ± 1.03 **.001**	14.9 ± 2.31 **<.001**
$LVID_d$/AAD	–	1.667 ± 0.164	**.007**	1.675 ± 0.182 .98	1.818 ± 0.143 **.006**	**<.001**	1.747 ± 0.133 .52	1.798 ± 0.184 .15	1.977 ± 0.210 **<.001**
$LVID_d$ (500)	cm	11.1 ± 0.89	**.002**	11.3 ± 1.21 .58	12.1 ± 0.77 **.001**	**<.001**	11.3 ± 0.84 .91	12.8 ± 1.07 **<.001**	13.8 ± 1.65 **<.001**
$LVFW_d$	cm	2.6 ± 0.39	.33	2.5 ± 0.36	2.7 ± 0.41	.31	2.5 ± 0.23	2.8 ± 0.35	2.7 ± 0.46
IVS_s	cm	4.5 ± 0.43	.4	4.4 ± 0.49	4.4 ± 0.43	.26	4.8 ± 0.46	4.8 ± 0.40	4.8 ± 0.82
$LVID_s$	cm	6.9 ± 1.06	**.04**	7.2 ± 1.14 .55	7.7 ± 1.05 **.02**	**<.001**	7.0 ± 0.72 1	7.8 ± 1.19 .2	9.1 ± 2.11 **<.001**
$LVFW_s$	cm	4.7 ± 0.529	.29	4.5 ± 0.49	4.4 ± 0.75	.87	4.5 ± 0.40	4.7 ± 0.72	4.6 ± 0.60

TABLE 2 (Continued)

Variable	Unit	Healthy (mean ± SD)	Mitral regurgitation (mean ± SD) (*P* value post hoc test)			Aortic regurgitation (mean ± SD) (*P* value post hoc test)			
			P value *F*-test (ANOVA)	Trivial-mild	Moderate-severe	*P* value *F*-test (ANOVA)	Trivial-mild	Moderate	Severe
LV FS	%	40 ± 6.4	.78	40 ± 6.7	39 ± 6.5	.76	42 ± 5.4	42 ± 6.9	39 ± 7.0
RWT_d	–	0.487 ± 0.063	**.03**	0.457 ± 0.085 .22	0.433 ± 0.052 **.01**	**.01**	0.466 ± 0.061 .81	0.443 ± 0.066 .27	0.401 ± 0.106 **.004**
MWT_d	cm	2.8 ± 0.24	.45	2.7 ± 0.30	2.7 ± 0.32	.45	2.8 ± 0.26	2.9 ± 0.34	2.9 ± 0.38
LAD_{max}/ $LVID_d$	–	1.102 ± 0.088	.47	1.075 ± 0.088	1.104 ± 0.088	**<.001**	1.093 ± 0.084 0.99	1.009 ± 0.067 **0.01**	0.926 ± 0.067 **<.001**
Area measurements of LV size and function (2D echocardiography, long axis)									
$LVIA_d$	cm^2	185.6 ± 18.17	.21	187.8 ± 25.38	195.7 ± 15.51	**<.001**	197.4 ± 15.80 .41	212.8 ± 20.58 **.004**	233.4 ± 31.34 **<.001**
$LVIA_d/AAD^2$	–	3.861 ± 0.487	.14	3.726 ± 0.645	4.071 ± 0.452	.06	4.268 ± 0.523	3.836 ± 0.552	4.296 ± 0.628
$LVIA_d$ (500)	cm^2	170.5 ± 15.89	.09	169.2 ± 21.27	180.2 ± 14.53	**<.001**	176.1 ± 12.29 .77	192.8 ± 20.47 **.003**	209.8 ± 18.75 **<.001**
$LVIA_s$	cm^2	82.6 ± 13.13	**.01**	92.0 ± 16.77 .06	95.4 ± 16.88 **.01**	**<.001**	100.1 ± 16.93 **.03**	95.0 ± 11.85 .088	107.0 ± 23.39 **.0003**
LV FAC	%	56 ± 5.0	**.007**	51 ± 4.8 **.009**	52 ± 6.4 **.02**	.06	50 ± 5.9	55 ± 3.4	55 ± 5.9
$LAA_{max}/LVIA_d$	–	0.594 ± 0.060	**.007**	0.599 ± 0.071 .95	0.659 ± 0.091 **.006**	**<.001**	0.545 ± 0.045 .15	0.552 ± 0.056 .19	0.499 ± 0.076 **<.001**
Area measurements of LV size and function (2D echocardiography, short axis)									
$LVI_{sx}A_d$	cm^2	97.3 ± 15.34	**.001**	100.6 ± 16.81 .67	114.1 ± 14.15 **<.001**	**<.001**	104.3 ± 12.96 .81	123.8 ± 15.73 **.015**	160.6 ± 46.23 **<.001**
$LVI_{sx}A_d/AAD^2$	–	2.029 ± 0.391	**.004**	2.002 ± 0.407 .96	2.385 ± 0.356 **.006**	**<.001**	2.238 ± 0.328 .44	2.234 ± 0.379 .45	2.821 ± 0.520 **<.001**
$LVI_{sx}A_d$ (500)	cm^2	89.4 ± 13.89	**<.001**	90.6 ± 14.80 .93	105.0 ± 13.33 **<.001**	**<.001**	93.4 ± 11.01 .86	112.1 ± 14.33 **.001**	136.8 ± 24.80 **<.001**
$LVI_{sx}A_s$	cm^2	32.8 ± 9.51	**.01**	36.0 ± 10.26 .45	41.7 ± 10.96 **.007**	**<.001**	35.5 ± 7.12 .95	42.4 ± 9.91 .26	61.2 ± 30.38 **<.001**
LV_{sx} FAC	%	67 ± 5.9	.28	64 ± 7.2	64 ± 7.9	.43	66 ± 3.5	66 ± 5.1	63 ± 8.6
RWT_d A_{sx}	–	0.495 ± 0.063	**<.001**	0.462 ± 0.062 .11	0.424 ± 0.063 **<.001**	**<.001**	0.449 ± 0.037 .236	0.434 ± 0.052 .063	0.388 ± 0.106 **<.001**
MWT_d A_{sx}	cm	2.7 ± 0.21	**.04**	2.6 ± 0.25 .11	2.5 ± 0.32 **.04**	.59	2.6 ± 0.20	2.7 ± 0.27	2.7 ± 0.46

(Continues)

TABLE 2 (Continued)

Variable	Unit	Healthy (mean ± SD)	Mitral regurgitation (mean ± SD) (*P* value post hoc test)			Aortic regurgitation (mean ± SD) (*P* value post hoc test)			
			P value *F*-test (ANOVA)	Trivial-mild	Moderate-severe	*P* value *F*-test (ANOVA)	Trivial-mild	Moderate	Severe
Volumetric estimates of LV size and function (2D echocardiography)									
Modified (single plane) Simpson's (S) model of discs									
$LVIV_d$ S	mL	1497 ± 232.1	**.05**	1542 ± 337.0 .78	1692 ± 233.6 **.03**	**< .001**	1676 ± 233.2 .36	1887 ± 290.2 **.004**	2258 ± 489.5 **<.001**
$LVIV_d$ S/AD^3	–	4.504 ± 0.891	**.05**	4.332 ± 1.165 .76	5.094 ± 0.931 .09	**.01**	5.325 ± 0.941 .14	4.592 ± 1.022 .99	5.639 ± 1.287 **.008**
$LVIV_d$ S (500)	mL	1316 ± 195.3	**.01**	1316 ± 261.2 1	1493 ± 195.3 **.01**	**<.001**	1409 ± 161.1 .66	1630 ± 283.6 **.002**	1962 ± 318.9 **<.001**
$LVIV_s$ S	mL	431 ± 107.3	**.004**	524 ± 161.0 **.04**	565 ± 162.7 **.003**	**<.001**	600 ± 163.5 **.015**	542 ± 109.5 .1	660 ± 215.1 **<.001**
LV EF S	%	71 ± 4.9	**.003**	66 ± 5.7 **.003**	67 ± 6.3 **.02**	**.02**	64 ± 6.7 **.005**	71 ± 3.7 1	71 ± 5.5 1
SV S	mL	1065 ± 167.9	.14	1016 ± 222.0	1127 ± 124.2	**<.001**	1076 ± 153.1 1	1345 ± 223.2 **.003**	1598 ± 332.1 **<.001**
CO S	L/min	44.8 ± 11.72	.77	42.5 ± 13.70	44.7 ± 10.13	**<.001**	43.3 ± 9.24 .98	53.1 ± 12.70 .19	64.5 ± 13.42 **<.001**
CO S (500)	L/min	39.3 ± 8.72	.67	36.9 ± 11.13	38.9 ± 9.06	**<.001**	36.0 ± 7.15 .82	45.5 ± 10.21 .19	54.8 ± 8.62 **<.001**
Area length (AL) model									
$LVIV_d$ AL	mL	1566 ± 250.5	**.04**	1611 ± 350.1 .79	1774 ± 249.8 **.03**	**<.001**	1768 ± 253.9 .33	1992 ± 308.4 **.003**	2407 ± 532.3 **<.001**
$LVIV_d$ AL/AAD^3	–	4.710 ± 0.951	**.05**	4.528 ± 1.206 0.76	5.331 ± 0.944 .09	**.007**	5.616 ± 1.007 .12	4.839 ± 1.057 .98	6.009 ± 1.383 **.004**
$LVIV_d$ AL (500)	mL	1378 ± 210.9	**.01**	1374 ± 264.9 1	1565 ± 205.3 **.01**	**<.001**	1486 ± 177.3 .61	1720 ± 295.3 **.002**	2092 ± 353.8 **<.001**
$LVIV_s$ AL	mL	434 ± 115.6	**.004**	529 ± 160.7 **.044**	572 ± 167.6 **.003**	**<.001**	610 ± 187.8 **.025**	552 ± 118.9 .13	694 ± 243.1 **<.001**
LV EF AL	%	72 ± 5.0	**.004**	67 ± 5.3 **.004**	68 ± 6.4 **.02**	**.04**	66 ± 6.9 **.016**	72 ± 3.8 1	72 ± 5.7 .97
SV AL	mL	1132 ± 181.0	.13	1083 ± 228.6	1202 ± 140.0	**<.001**	1158 ± 152.6 .99	1440 ± 234.7 **.002**	1714 ± 348.6 **<.001**
CO AL	L/min	47.9 ± 12.41	.73	45.3 ± 14.06	47.8 ± 10.85	**<.001**	46.5 ± 9.18 .99	56.9 ± 13.71 .17	69.3 ± 14.31 **<.001**
CO AL (500)	L/min	41.9 ± 9.195	.62	39.2 ± 11.28	41.4 ± 9.61	**<.001**	39.1 ± 5.72 .86	48.7 ± 10.91 .15	58.7 ± 9.05 **<.001**

TABLE 2 (Continued)

Variable	Unit	Healthy (mean ± SD)	Mitral regurgitation (mean ± SD) (*P* value post hoc test)			Aortic regurgitation (mean ± SD) (*P* value post hoc test)			
			P value *F*-test (ANOVA)	Trivial-mild	Moderate-severe	*P* value *F*-test (ANOVA)	Trivial-mild	Moderate	Severe
Bullet (B) model									
$LVIV_d$ B	mL	1525 ± 290.3	**.02**	1575 ± 309.8 .75	1751 ± 233.3 **.01**	**<.001**	1663 ± 185.7 .72	2006 ± 325.1 **.004**	2448 ± 660.4 **<.001**
$LVIV_d$ B/AAD^3	–	4.600 ± 1.091	**.04**	4.428 ± 1.176 .8	5.291 ± 0.996 .07	**.009**	5.290 ± 0.916 .33	4.878 ± 1.127 .87	6.022 ± 1.285 **.003**
$LVIV_d$ B (500)	mL	1342 ± 246.4	**.01**	1349 ± 264.7 .99	1549 ± 229.8 **.01**	**<.001**	1402 ± 160.9 .93	1728 ± 273.3 **.003**	2120 ± 449.4 **<.001**
$LVIV_s$ B	mL	373 ± 127.4	**.03**	417 ± 141.1 .39	481 ± 138.5 **.013**	**<.001**	435 ± 85.5 0.64	504 ± 138.1 0.07	645 ± 236.7 **<0.001**
LV EF B	%	76 ± 4.8	.12	74 ± 5.6	73 ± 6.9	.6	74 ± 3.1	75 ± 4.4	74 ± 6.3
SV B	mL	1151 ± 194.4	.11	1158 ± 232.6	1269 ± 186.0	**<.001**	1227 ± 124.6 .85	1502 ± 226.8 **.004**	1803 ± 505.5 **<.001**
CO B	L/min	47.8 ± 12.33	.77	48.7 ± 14.52	50.7 ± 14.43	**<.001**	49.3 ± 9.03 .99	58.2 ± 13.44 .14	73.2 ± 20.82 **<.001**
CO B (500)	L/min	41.7 ± 8.29	.5	41.4 ± 11.48	45.0 ± 13.87	**<.001**	41.4 ± 6.17 1	49.8 ± 10.40 .09	63.4 ± 15.03 **<.001**

Note: Significant differences between groups are marked in bold. For detailed explanation of echocardiographic indices see Appendix S1.
Abbreviations: BWT, body weight; HR, heart rate; n, number of horses; SD, standard deviation.

TABLE 3 Intraobserver and interobserver measurement variability of variables used for measurements of LV size and function

		Intraobserver variability			Interobserver variability		
Variables	**Unit**	**Mean ± SD**	**CV (%)**	**RC**	**Mean ± SD**	**CV (%)**	**RC**
Heart rate							
HR	bpm	46 ± 8.6	0.8	1	46 ± 8.6	1.1	1
Aortic dimensions							
AAD	cm	7.0 ± 0.84	3.3	0.6	6.8 ± 0.81	3.7	0.7
Measurements of LA size							
LAD_{max}	cm	12.6 ± 1.21	2.1	0.7	12.4 ± 1.23	3.1	1.1
LAD_{max}/AAD	–	1.827 ± 0.162	5.0	0.252	1.831 ± 0.160	4.7	0.237
LAD_{max} (500)	cm	12.1 ± 0.69	2.1	0.7	11.9 ± 0.78	3.0	1.0
LAA_{max}	cm^2	108.1 ± 18.12	3.0	9.0	104.2 ± 18.85	7.7	22.1
LAA_{max}/AAD^2	–	2.289 ± 0.441	10.8	0.684	2.268 ± 0.408	8.4	0.530
LAA_{max} (500)	cm^2	98.7 ± 8.46	2.8	7.8	95.1 ± 9.80	7.3	19.1
Linear measurements of LV size and function (M-mode, short axis at chordal level)							
IVS_d	cm	3.0 ± 0.41	4.7	0.4	3.1 ± 0.40	6.2	0.5
$LVID_d$	cm	11.9 ± 2.29	0.9	0.3	11.9 ± 2.30	2.0	0.7
$LVID_d/AAD$	–	1.794 ± 0.549	5.5	0.274	1.741 ± 0.240	5.7	0.274
$LVID_d$ (500)	cm	11.3 ± 1.83	0.8	0.2	11.4 ± 1.86	2.0	0.6
$LVFW_d$	cm	2.6 ± 0.40	5.9	0.4	2.6 ± 0.42	4.7	0.3
IVS_s	cm	4.4 ± 0.62	3.6	0.4	4.4 ± 0.64	3.2	0.4
$LVID_s$	cm	7.1 ± 1.51	2.2	0.4	7.1 ± 1.43	1.9	0.4
$LVFW_s$	cm	4.6 ± 0.76	2.3	0.3	4.5 ± 0.75	2.4	0.3
LV FS	%	40 ± 7.4	3.2	4	40 ± 6.9	3.3	4
RWT_d	–	0.485 ± 0.075	4.1	0.055	0.494 ± 0.088	4.9	0.067
MWT_d	cm	2.8 ± 0.35	3.9	0.3	2.9 ± 0.37	4.4	0.4
$LAD_{max}/LVID_d$	–	1.082 ± 0.145	2.2	0.067	1.066 ± 0.142	3.5	0.102
Area measurements of LV size and function (2D echocardiography, long axis)							
$LVIA_d$	cm^2	189.4 ± 44.92	2.8	14.8	183.4 ± 44.51	6.3	32.1
$LVIA_d/AAD^2$	–	3.960 ± 0.744	7.9	0.864	3.942 ± 0.643	5.6	0.612
$LVIA_d$ (500)	cm^2	171.1 ± 27.55	5.3	25.3	165.7 ± 27.50	6.3	29.0
$LVIA_s$	cm^2	88.7 ± 27.43	5.0	12.3	87.0 ± 28.32	7.3	17.7
LV FAC	%	54 ± 6.6	2.6	4	53 ± 6.7	2.8	4
$LAA_{max}/LVIA_d$	–	0.585 ± 0.096	4.2	0.068	0.583 ± 0.102	3.4	0.055
Area measurements of LV size and function (2D echocardiography, short axis)							
$LVI_{sx}A_d$	cm^2	102.0 ± 35.75	5.5	15.4	101.6 ± 37.18	3.6	10.0
$LVI_{sx}A_d/AAD^2$	–	2.112 ± 0.567	10.3	0.605	2.156 ± 0.547	9.3	0.553
$LVI_{sx}A_d$ (500)	cm^2	92.6 ± 26.65	5.6	14.5	92.0 ± 27.47	3.6	9.2
$LVI_{sx}A_s$	cm^2	35.4 ± 14.83	4.5	4.5	36.3 ± 15.68	3.1	3.1
LV_{sx} FAC	%	65 ± 8.60	3.3	6	64 ± 9.3	3.6	6
MWT_d A_{sx}	cm	2.7 ± 0.30	4.1	0.3	3.0 ± 0.37	10.2	0.8
RWT_d A_{sx}	–	0.495 ± 0.082	4.9	0.068	0.544 ± 0.093	10.6	0.160
Volumetric estimates of LV size and function (2D echocardiography)							
Modified (single plane) Simpson's (S) model of discs							
$LVIV_d$ S	mL	1632 ± 666.0	4.1	186	1542 ± 644.6	10.2	434
$LVIV_d$ S/AAD^3	–	4.808 ± 1.339	11.8	1.574	4.737 ± 1.175	8.5	1.118
$LVIV_d$ S (500)	mL	1400 ± 432.0	4.7	183	1325 ± 423.2	10.4	381
$LVIV_s$ S	mL	504 ± 256.1	7.8	109	483 ± 251.9	12.9	173

TABLE 3 (Continued)

		Intraobserver variability			Interobserver variability		
Variables	**Unit**	**Mean ± SD**	**CV (%)**	**RC**	**Mean ± SD**	**CV (%)**	**RC**
LV EF S	%	70 ± 6.6	1.9	4	70 ± 6.4	2.0	4
SV S	mL	1128 ± 454.1	3.9	122	1059 ± 434.9	10.0	293
CO S	L/min	49.3 ± 16.63	3.6	4.9	46.4 ± 15.88	10.0	12.9
CO S (500)	L/min	42.3 ± 10.17	4.2	4.9	39.8 ± 9.73	10.1	11.1
Area length (AL) model							
$LVIV_d$ AL	mL	1710 ± 725.8	4.5	215	1627 ± 708.5	10.0	450
$LVIV_d$ AL/AAD^3	–	5.036 ± 1.478	12.3	1.719	4.981 ± 1.295	8.6	1.188
$LVIV_d$ AL (500)	mL	1467 ± 475.5	5.0	202	1396 ± 469.9	10.3	397
$LVIV_s$ AL	mL	516 ± 276.3	8.0	114	495 ± 278.7	13.5	186
LV EF AL	%	71 ± 6.6	1.8	3	495 ± 278.7	2.0	4
SV AL	mL	1194 ± 495.1	4.5	148	1131 ± 474.5	9.3	291
CO AL	L/min	52.1 ± 17.67	4.1	6.0	49.4 ± 17.00	9.3	12.7
CO AL (500)	L/min	44.6 ± 10.67	4.4	5.4	42.4 ± 10.34	9.4	11.0
Bullet (B) model							
$LVIV_d$ B	mL	1590 ± 682.6	6.3	279	1563 ± 689.0	5.1	220
$LVIV_d$ B/AAD^3	–	4.792 ± 1.752	16.2	2.146	4.863 ± 1.513	11.7	1.579
$LVIV_d$ B (500)	mL	1373 ± 473.7	6.6	252	1347 ± 468.1	5.0	188
$LVIV_s$ B	mL	413 ± 208.5	7.5	85	427 ± 222.6	3.1	37
LV EF B	%	74 ± 7.9	2.7	6	73 ± 8.5	2.5	5
SV B	mL	1176 ± 526.1	8.1	264	1137 ± 516.9	7.3	230
CO B	L/min	53.7 ± 21.75	9.0	13.3	52.0 ± 22.06	8.2	11.8
CO B (500)	L/min	45.6 ± 13.70	9.0	11.4	44.0 ± 13.63	7.9	9.6

Note: For detailed explanation of echocardiographic indices see Appendix S1.
Abbreviations: CV, coefficient of variation; RC, repeatability coefficient; SD, standard deviation.

concept that echocardiographic indices of LV size, to different extents, can identify significant LV enlargement in horses with moderate and severe mitral and aortic regurgitation, respectively (Table 2).

Independent of the geometric model used, SV was significantly altered by moderate and severe AR and CO was significantly altered by severe AR, but neither SV nor CO was significantly altered by MR, likely to be explained by the lesser degree of LV volume overload (ie, preload, represented by end-diastolic LV dimensions) seen in the group of horses with MR compared to horses with AR in this study. The ejection phase indices that are primarily based on LV short-axis measurements (ie, LV FS, LV_{sx} FAC, and LV EF B) were not able to detect differences between healthy horses and horses with valvular disease. Conversely, those that are primarily based on LV long-axis measurements (ie, LV FAC, LV EF S, and LV EF AL) allowed to detect disease-related differences. This suggests, in agreement with recommendations published in the human literature,[36-41] that long-axis motion of the myocardium is critical in health and disease and should be considered when assessing cardiac mechanical function in horses.

Overall, these findings support the complementary use of area-based long-axis measurements of LV size and function and respective volumetric estimates, in addition to the traditional linear short-axis measurements in Warmblood horses with cardiac disease. Table 4 and Supporting Information Figures S2 and S5 show that the single-plane Simpson's model and the area-length model provide very similar estimates of LV size and function, which can be explained by the fact that these models are both based on an LV long-axis tracing (see Appendix S1), whereas the bullet model also includes a measurement of the LV short-axis area and therefore results in slightly different measurements. The authors routinely use the single-plane Simpson's model of discs to calculate volumetric estimates of LV size and function. However, the results of this study do not justify preferring 1 volumetric model over another. A previous study on volumetric echocardiography methods for cardiac output measurement in healthy adult horses reported that Simpson's, area-length, and bullet models all provided better agreement with lithium dilution than other methods (including Teichholz and Cubic methods based on linear M-mode measurements and Doppler interrogation of blood flow in the left-ventricular outflow tract), but also was not able to identify clear advantages of 1 model over another.[13]

Critical appraisal of the measurement variability (Table 3 and Supporting Information Table S1) indicates an overall low variability for all variables of LV size and function that were under investigation. Indexing variables of end-diastolic LV dimensions to AAD introduced additional measurement variability, again suggesting that this may not

TABLE 4 Proportion (%) of horses in which different methods of measurement obtained during a single examination revealed discordant results concerning left ventricular dimensions and systolic function

Variables indicate normal LV dimensions and systolic function	Variables indicate LV dimensions and systolic function outside normal limits	All horses (healthy and valvular regurgitation) n = 100		κ_w *(all horses)*	Horses with valvular regurgitation n = 70		κ_w *(valvular regurgitation)*
$LVID_d$ (500)	$LVIA_d$ (500)	8/79	10.1%	0.602	7/49	14.3%	0.59
	$LVI_{sx}A_d$ (500)	3/80	3.8%	0.719	2/50	4.0%	0.725
	$LVIV_d$ S (500)	8/79	10.1%	0.669	7/49	14.3%	0.659
	$LVIV_d$ AL (500)	7/79	8.9%	0.656	6/49	12.2%	0.647
	$LVIV_d$ B (500)	6/79	7.6%	0.716	4/49	8.2%	0.744
$LVIA_d$ (500)	$LVID_d$ (500)	5/76	6.6%	0.602	5/47	10.6%	0.59
	$LVI_{sx}A_d$ (500)	3/76	3.9%	0.661	3/47	6.4%	0.614
	$LVIV_d$ S (500)	5/76	6.6%	0.784	5/47	10.6%	0.752
	$LVIV_d$ AL (500)	4/76	5.3%	0.777	4/47	8.5%	0.745
	$LVIV_d$ B (500)	5/76	6.6%	0.718	4/47	8.5%	0.704
$LVI_{sx}A_d$ (500)	$LVID_d$ (500)	5/82	6.1%	0.719	5/53	9.4%	0.725
	$LVIA_d$ (500)	8/81	9.9%	0.661	8/52	15.4%	0.614
	$LVIV_d$ S (500)	8/81	9.9%	0.727	8/52	15.4%	0.686
	$LVIV_d$ AL (500)	8/81	9.9%	0.653	8/52	15.4%	0.601
	$LVIV_d$ B (500)	6/81	7.4%	0.777	5/52	9.6%	0.774
$LVIV_d$ S (500)	$LVID_d$ (500)	3/74	4.1%	0.669	3/45	6.7%	0.659
	$LVIA_d$ (500)	3/74	4.1%	0.784	3/45	6.7%	0.752
	$LVI_{sx}A_d$ (500)	1/74	1.4%	0.727	1/45	2.2%	0.686
	$LVIV_d$ AL (500)	0/74	0.0%	0.945	0/45	0.0%	0.936
	$LVIV_d$ B (500)	5/74	6.8%	0.664	4/45	8.9%	0.634
$LVIV_d$ AL (500)	$LVID_d$ (500)	4/76	5.3%	0.656	4/47	8.5%	0.647
	$LVIA_d$ (500)	4/76	5.3%	0.777	4/47	8.5%	0.745
	$LVI_{sx}A_d$ (500)	3/76	3.9%	0.653	3/47	6.4%	0.601
	$LVIV_d$ S (500)	2/76	2.6%	0.945	2/47	4.3%	0.936
	$LVIV_d$ B (500)	7/76	9.2%	0.595	6/47	12.8%	0.555
$LVIV_d$ B (500)	$LVID_d$ (500)	3/76	3.9%	0.716	3/48	6.3%	0.744
	$LVIA_d$ (500)	5/76	6.6%	0.718	5/48	10.4%	0.704
	$LVI_{sx}A_d$ (500)	1/76	1.3%	0.777	1/48	2.1%	0.774
	$LVIV_d$ S (500)	7/76	9.2%	0.664	7/48	14.6%	0.634
	$LVIV_d$ AL (500)	7/76	9.2%	0.595	7/48	14.6%	0.555
RWT_d	RWT_d A_{sx}	8/92	8.7%	0.363	7/62	11.3%	0.369
RWT_d A_{sx}	RWT_d	4/88	4.5%	0.363	4/59	6.8%	0.369
MWT_d	MWT_d A_{sx}	6/90	6.7%	0.476	6/60	10.0%	0.464
MWT_d A_{sx}	MWT_d	4/88	4.5%	0.476	4/58	6.9%	0.464
LV FS	LV FAC	9/95	9.5%	−0.03	9/67	13.4%	−0.024
	LV_{sx} FAC	4/96	4.2%	0.233	4/68	5.9%	0.32
	LV EF S	8/95	8.4%	−0.029	8/67	11.9%	−0.023
	LV EF AL	10/95	10.5%	−0.031	10/67	14.9%	−0.025
	LV EF B	6/95	6.3%	0.178	6/67	9.0%	0.233
LV FAC	LV FS	3/89	3.4%	−0.03	1/59	1.7%	−0.024
	LV_{sx} FAC	2/88	2.3%	0.279	2/58	3.4%	0.266
	LV EF S	0/89	0.0%	0.937	0/59	0.0%	0.935

TABLE 4 (Continued)

Variables indicate normal LV dimensions and systolic function	Variables indicate LV dimensions and systolic function outside normal limits	All horses (healthy and valvular regurgitation) n = 100		κ_w (all horses)	Horses with valvular regurgitation n = 70		κ_w (valvular regurgitation)
	LV EF AL	1/89	1.1%	0.943	1/59	1.7%	0.941
	LV EF B	2/89	2.2%	0.6	2/59	3.4%	0.588
LV_{sx} FAC	LV FS	2/94	2.1%	0.233	0/64	0.0%	0.32
	LV FAC	7/93	7.5%	0.279	7/63	11.1%	0.266
	LV EF S	6/93	6.5%	0.307	6/63	9.5%	0.295
	LV EF AL	8/93	8.6%	0.255	8/63	12.7%	0.24
	LV EF B	3/93	3.2%	0.717	3/63	4.8%	0.712
LV EF S	LV FS	3/90	3.3%	−0.029	1/60	1.7%	−0.023
	LV FAC	1/90	1.1%	0.937	1/60	1.7%	0.935
	LV_{sx} FAC	2/89	2.2%	0.307	2/59	3.4%	0.295
	LV EF AL	2/90	2.2%	0.88	2/60	3.3%	0.876
	LV EF B	3/90	3.3%	0.504	3/60	5.0%	0.49
LV EF AL	LV FS	3/88	3.4%	−0.031	1/58	1.7%	−0.025
	LV FAC	0/88	0.0%	0.943	0/58	0.0%	0.941
	LV_{sx} FAC	2/87	2.3%	0.255	2/57	3.5%	0.24
	LV EF S	0/88	0.0%	0.88	0/58	0.0%	0.876
	LV EF B	2/88	2.3%	0.559	2/58	3.4%	0.545
LV EF B	LV FS	2/91	2.2%	0.178	0/61	0.0%	0.233
	LV FAC	4/91	4.4%	0.6	4/61	6.6%	0.588
	LV_{sx} FAC	0/90	0.0%	0.717	0/60	0.0%	0.712
	LV EF S	4/91	4.4%	0.504	4/61	6.6%	0.49
	LV EF AL	5/91	5.5%	0.559	5/61	8.2%	0.545

Note: Weighted Kappa (κ_w) quantifies the method agreement: >0.75 = excellent (shaded in green), 0.4-0.75 = fair to good (shaded in yellow), 0-0.39 = poor (shaded in orange), <0 = worse (shaded in red). For detailed explanation of echocardiographic indices see Appendix S1.

be the preferred method for allometric scaling of variables (also see above). Both intraobserver and interobserver measurement variabilities were lowest for linear variables compared to area-based variables and volumetric estimates of LV size and function, respectively. This can be explained by the additional dimensions influencing measurements and geometric estimates reported. Nonetheless, all CV were judged to be sufficiently low for the respective variables to be used in clinical routine. In any case, alterations in variables of LV size and function would need to be sufficiently large (ie, larger than the respective RC) to be reliably detected in individual horses. Because of the retrospective study design, this study only investigated the measurement variability based on a single dataset measured repeatedly, mostly influenced by operator-related and algorithm-related factors. The day-to-day biological variability that would also affect the variables in a clinical setting over time will have to be quantified in future studies.

With the lack of a gold standard, this study does not allow quantifying accuracy of the respective variables or proving the superiority of area-based measurements and volumetric estimates of LV size over unidimensional variables. However, the results indicate that agreement of different indices for detection of abnormal end-diastolic LV dimensions was fair to excellent for all variables (Table 4 and Supporting Information Figures S1 and S2). There was fair to good agreement between MWT_d and MWT_d A_{sx} but poor agreement between RWT_d and RWT_d A_{sx} (Table 4 and Supporting Information Figure S3). Most strikingly, agreement between LV FS and all other ejection phase indices was poor or worse and agreement between LV_{sx} FAC and LV FAC, LV EF S and LV EF AL, respectively, was poor (Table 4 and Supporting Information - Figure S4 and S5). This again supports the contention that variables describing the short-axis motion of the LV do not adequately reflect long-axis motion nor describe overall LV mechanical function.[36-41] Excellent agreement was seen between LV EF S and LV EF AL, respectively, and LV FAC, and between LV EF S and LV EF AL, most likely representing the fact that these variables are all mathematically related. Fair to good agreement was seen between LV EF B and all other area-based and volumetric ejection phase indices.

However, even for variables with fair to excellent agreement, the use of different variables may lead to discordant conclusions with regard to the presence of LV enlargement or systolic dysfunction in individual horses (Table 4 and Supporting Information Figures S1-S5). This can likely be explained by inherent measurement variability and by the fact that variables represent different uniplanar or biplanar dimensions of an asymmetrical 3-dimensional structure that can enlarge in a multidirectional fashion.[16,42] Although on a theoretical basis the use of area-based and volumetric variables might be preferable, the results of this study do not unconditionally support this assumption. The results however strongly suggest that in addition to subjective assessment of LV size and function, a variety of different variables, including conventional linear measurements as well as area-based measurements or volumetric estimates, should be jointly considered for diagnosing and documenting LV dilation and systolic dysfunction in horses.

In conclusion, this study defines reference intervals for a variety of uni-, 2-, and 3-dimensional echocardiographic indices of LV size and function in Warmblood horses and demonstrates that measurement variability is sufficiently low for clinical use of all variables. Allometric scaling appears to be effective and practical to correct diastolic LV dimensions and CO for differences in body size within breed. In clinical practice, systolic LV dimensions, variables representing wall thickness and fractional changes of LV internal dimensions may not need correction due to minor influence of BWT on these variables. Scaling to a BWT of 500 kg is preferred over aortic indexing for assessing diastolic LV size. Various echocardiographic indices can result in different conclusions with regard to identification of LV enlargement and systolic dysfunction in horses with mitral and aortic regurgitation, suggesting that assessment of LV size and function should be based on an integrative approach of subjective evaluation and joint assessment of a combination of multiple uni- and multi-dimensional measurements and indices. Importantly, variables that reflect LV long-axis motion should be included for comprehensive assessment of LV function.

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CONFLICT OF INTEREST DECLARATION

Colin Schwarzwald serves as Associate Editor for the Journal of Veterinary Internal Medicine. He was not involved in review of this manuscript.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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